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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 14 NOVEMBER 1956 No. 11

SOME ASPECTS OF NUTRITIONAL RESEARCH IN SOUTH AFRICA*

In South Africa, living side by side with the white population, is a large Bantu population which is observable in various stages of sophistication of diet and manner of life. Speaking generally, the diet of these people is probably adequate in calories and total protein, is low in animal protein, fat, and certain mineral salts and vitamins, but is high in carbohydrate and crude fibre. This situation is of interest for at least three reasons. First, because there is evidence that such a diet, directly or indirectly, gives rise to or promotes various departures from "normal," the precise causation and significance of which are still largely obscure. Second, a population consuming such a diet provides a "touchstone" against which may be tested many of the orthodox concepts on (1) recommended allowances of nutrients, and (2) physiologic functions such as lactation and hemopoiesis. Third, the Bantus appear to suffer very little from a number of important diseases in which the nutritional factor is variously implicated. A few examples in these three fields of research will now be given, illustrations being selected mainly from investigations undertaken by the writer and associate workers in Johannesburg.

(1) Departures from "Normal" in the Bantu

Blood. No difference has been found between newborn Bantu and white babies with regard to mean values for fractionated serum proteins, serum cholesterol and esters, and various liver function tests, yet highly significant differences are found between the respective means for the mothers of both races (I. Bersohn, S. Wayburne, H. Hirsch, and C. D. Sussman, *South African J. Clin. Sci.* **5,** *35 (1954)*; Bersohn and Wayburne, *Am. J. Clin. Nutrition* **4,** *117 (1956)*).

Breast Milk. Bantu mothers consuming a high maize diet, including an apparently adequate intake of niacin (although presumably much of it is in "bound" form) have a breast milk concentration of this vitamin less than half the mean value found in white mothers. This abnormality, not found in Bantu mothers consuming a Europeanized diet, is not believed to be of nutritional consequence (A. R. P. Walker, *Nature* **173,** *405 (1954)*).

Urine. From balance observations, we have noted that volumes of Bantu urine collected over twenty-four hours are much greater than corresponding volumes from white subjects. This phenomenon, which has often been observed in white subjects on high carbohydrate diets, has not yet been adequately explained (R. A. McCance and E. M. Widdowson, *Med. Res. Council (London) Special Report Series No. 254 (1946)*; D. A. Smith and M. F. A. Woodruff, *Ibid. No. 274 (1951)*).

Feces. Adult Bantus void daily up to four times the amount of feces compared with white people on a white bread dietary. Using carmine as a marker, the time of passage with the Bantu is found to be shorter, often about one-half that of white subjects. Further, Bantu feces occlude as much as 50 per cent more gas compared with the feces of white persons. Combining these observations, it is clear that *a given volume* of fecal material in the Bantu may pass through the digestive tract many times faster than in white subjects. The effect of this rapid passage on synthesis, breakdown, absorption, resorption, microflora population, morphology of the intestinal tract, etc., has been virtually unstudied. Concerning the digestibility of nutrients, on very high

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cereal diets, R. A. McCance and C. M. Walsham (*Brit. J. Nutrition* **2,** *26 (1948)*) stated that protein nitrogen is completely digested. Excluding obviously unchewed food (*e.g.*, whole maize and beans) we have not found undigested starch in adult Bantu feces, and not always in the diarrheal feces of infants with kwashiorkor. In regard to fat, large amounts of fat, up to 13 g. per day, are voided when the cereal intake is very high (apparent digestibility of fat 49 per cent); such fecal fat we believe to be mainly of endogenous origin, and *not* composed of unabsorbed fat (A. R. P. Walker, *Nature* **164,** *825 (1949)*; Walker and U. B. Arvidsson, *South African J. Sci.* **47,** *267 (1951)*).

(2) The Bantu as a "Touchstone" for Orthodox Beliefs in Nutrition

Calcium. The calcium intake of the Bantu is about one third to one half of that usually recommended. The phosphorus intake, much of it in the form of phytate phosphorus, is high. Moreover, where cereals are the almost exclusive source of calories, the calcium: phosphorus ratio is as "unfavorable" as 1:10. The amount of vitamin D ingested is almost nil. There is, of course, plenty of sunlight, although some believe that the dark skin of the Bantu militates against the production of vitamin D from ultraviolet irradiation. To determine the extent to which these people are handicapped by low calcium intake, we have investigated various aspects of calcium metabolism—calcium balance studies, the role of phytic acid phosphorus (A. R. P. Walker, *Lancet* **II,** *244 (1951)*), serum calcium levels (Walker, U. B. Arvidsson, and W. M. Politzer, *South African Med. J.* **28,** *48 (1954)*), the composition and histology of bone (Walker and Arvidsson, *Metabolism* **3,** *385 (1954)*; J. Higginson, *Ibid.* **3,** *392 (1954)*), physical bone density, studies on the incidence of rickets in urban and rural areas, the roles of calcium in influencing growth and in affecting the development of rickets (Walker, *Am. J. Clin. Nutrition* **2,** *265 (1954)*; *Ibid.* **3,** *114 (1955)*; *Nutrition Reviews* **14,** *36 (1956)*), liability to fractures, and certain comparative radiologic studies. Briefly, in none of these respects can we say precisely where these people are stigmatized by their low intake of calcium. In strong contrast to other nutrients, there appears to be no evidence in man of any syndrome caused *solely* by calcium deficiency or remediable by calcium supplementation *only*. Is it not possible that in different parts of the world, diets which support and maintain life contain enough calcium for satisfactory bone formation and for the other physiologic functions involving the element?

Ascorbic Acid (Vitamin C). Knowing the unsatisfactory composition of the foods used by Bantu mothers in weaning their children, there is no difficulty in forecasting the outcome, which varies from mild malnutrition to the severest forms of kwashiorkor (A. R. P. Walker, D. C. Fletcher, E. S. P. Strydom, and M. Andersson, *Brit. J. Nutrition* **9,** *38 (1955)*). Predictable stigmata, *e.g.*, retarded growth, edema, dermatosis, etc., are in evidence, yet one deficiency disease, namely, infantile scurvy, is inexplicably absent. We have frequently found that the intake of vitamin C, determined from examination of food prepared by Bantu mothers for their babies, is almost nil, yet clinical and radiologic observations on groups of such subjects have provided no evidence of scurvy. Moreover, plasma concentrations of vitamin C, even in infants with kwashiorkor, have been found to be consistent with an absence of scurvy. Hence, either all Bantu infants have access to sources of vitamin C which neither ourselves nor Bantu social workers are aware of, which we disbelieve, or we are driven to consider that Bantu infants under the conditions of cereal "pap" intake, are able to synthesise vitamin C (Andersson, Walker, and H. C. Falcke, *Ibid.* **10,** *101 (1956)*).

Lactation. Despite habitual consumption of a diet which, according to current beliefs,

would not be expected to favor normal lactation, Bantu mothers usually produce an abundance of milk and breast-feed frequently for protracted periods. Evidence indicates that their milk has a satisfactory chemical composition; furthermore, since Bantu and European babies when exclusively breast fed are comparable clinically at 6 months, confirmation is provided that Bantu breast milk is adequate in quantity as well as quality. In the face of this evidence it is questionable whether dietary deficiency can be invoked to explain the failure of the large proportion of white mothers to lactate satisfactorily (A. R. P. Walker, U. B. Arvidsson, and W. L. Draper, *Lancet* **I,** *317 (1952)*; *Tr. Roy. Soc. Trop. Med. Hyg.* **48,** *395 (1954)*). Among the Bantus, the induction of a flow of milk from virgins has been claimed, as well as the capacity to lactate even beyond the menopause (P. J. Greenway, *East African Med. J.* **13,** *346 (1937)*). At present we are carrying out analysis of "grandmothers' " breast milk. In view of these observations, we have to admit how incomplete is our knowledge of the factors, dietary and non-dietary, which influence lactation.

Hemopoiesis. The factors listed as being important in hemopoiesis are well known. Among the Bantus, their excessive iron intake (as much as 200 mg. per day) results in widespread siderosis, elevated serum iron concentrations, and a low incidence of hypochromic anemia (A. R. P. Walker and U. B. Arvidsson, *Tr. Roy. Soc. Trop. Med. Hyg.* **47,** *536 (1953)*; J. Higginson, T. Gerritsen, and Walker, *Am. J. Path.* **29,** *779 (1953)*). An interesting question is, what happens to these people, with their high and irreducible iron stores, during episodes of gross malnutrition or undernutrition? Does the anemia to be expected develop or is it hindered? Investigations indicate that satisfactory hemoglobin levels are often maintained, even in grossly malnourished pellagrins (Walker, *South African J. Lab. Clin. Med.* **1,** *36 (1955)*). Such observations confirm the high priority given by the body for blood production; they also suggest that, *provided the iron reserves be excessively high,* the other dietary hemopoietic factors are of much less importance.

(3) Research in Diseases in Which Diet is Believed to be an Etiologic Factor

Among the Bantu, certainly, there is a high incidence of liver disease, and various deficiency diseases are common. At the same time it must be pointed out that the Bantus rarely suffer from a large number of diseases which exact a high toll of mortality and morbidity among western peoples. Such diseases include appendicitis, gall stones, peptic ulcer, certain types of cancer, and atherosclerosis and coronary artery disease. There is more than suggestive evidence that the pattern of diet consumed by the Bantu is a strong etiologic factor in the prevention of these diseases, and that when these people adopt European food habits, the "protection" is largely lost.

Appendicitis. Among Bantu mineworkers, appendicitis is *one-eightieth* as common as among a somewhat comparable population, namely, American wartime Naval personnel. It may be apposite to quote a well known British surgeon, Zachary Cope, as maintaining that our present knowledge of the etiology of appendicitis is little in advance of what Fitz taught in 1880 (V. Z. Cope, *Brit. Med. J.* **1,** *1242 (1950)*).

Gall Stones. From a series of about 9000 consecutive necropsies on Bantus and local Europeans, the incidence of cholelithiasis was found to be 2.04 and 13.52 per cent respectively (B. J. P. Becker and C. B. Chatgidakis, *South African J. Clin. Sci.* **3,** *13 (1952)*).

Atheroma and Coronary Artery Disease. Published studies (A. R. P. Walker and U. B. Arvidsson, *J. Clin. Invest.* **33,** *1358 (1954)*) have shown that serum cholesterol concentrations are low and rise only slightly with age in the Bantu compared with white subjects; furthermore, the more severe

complications of atherosclerosis in the Bantu are only about one-tenth as common as among comparable groups of Europeans (J. Higginson and W. J. Pepler, *Ibid.* **33,** *1366 (1954)*; *Nutrition Reviews* **13,** *138 (1955)*). The correlation of low fat intake, low serum cholesterol concentration and low incidence of atherosclerosis is indisputable. Nevertheless, we are hesitant over attaching undue importance to the fat intake aspect, and believe it to be premature to go beyond correlating the *total* pattern of Bantu diet with atherosclerosis. In any case, it should be borne in mind that a low fat intake is but one feature of the Bantu diet, and that these people are relatively free from several diseases other than atherosclerosis (Walker, *Lancet* **I,** *565 (1955)*). The general observation that the mean serum cholesterol concentration of elderly Bantus does not rise beyond that of 20 to 30 year old white subjects is paralleled by similar evidence. Thus Bersohn and associate workers have shown that the electrophoretic lipoprotein pattern of elderly Bantus is even "younger" than that of young white subjects. Further, Miss M. Andersson, by determining the chemical composition of a large series of aortas from Bantu and white subjects of different age groups, has shown that, speaking generally, Bantu aortas retain their youth almost perennially, and scarcely exceed in vascular age those of young white subjects.

Conclusion

The few examples given serve to illustrate the wealth of research material available and the wide scope of the fundamental studies that can be undertaken in South Africa. We have no doubt that the answer to many nutritional and disease problems will be afforded by intensive study of the nonwhite populations in this El Dorado of medical research, for in this country we are particularly fortunate in being able to study the Bantu in all stages of transition, from the wandering primitive living off the land in the fastness of the Kalahari Desert, to the fully Europeanized urban dandy, with his zest for Wild West shows, and his partiality to white bread, sugar, and coca cola.

ALEXANDER R. P. WALKER,
Head, Human Biochemistry Research Unit, South African Institute for Medical Research, Johannesburg, and the Council for Scientific and Industrial Research

DAILY NITROGEN REQUIREMENTS OF ADULT MAN

The minimal daily nitrogen requirement of man has been studied in the past with diets containing intact protein (*see Nutrition Reviews* **4,** *264 (1946)*; **13,** *42 (1955)*). Values obtained with such diets may be high because of unnecessary excesses of one or more of the amino acids or because of poor or inefficient utilization of certain dietary proteins.

These objections have been overcome by recent preliminary work of W. C. Rose and R. L. Wixom (*J. Biol. Chem.* **217,** *997 (1955)*), who studied the minimum nitrogen intake of adult man with diets containing purified amino acids as the chief source of nitrogen. The experimental conditions were similar to those described in studies on the quantitative amino acid requirements of man carried out by Rose and co-workers (*see Nutrition Reviews* **14,** *232 (1956)*).

Two normal young men, weighing 68 and 81 kg., were used as subjects. They received highly purified diets containing twice the minimal levels of the eight essential amino acids. Initially additional nitrogen was supplied as urea and glycine to provide a total of 10 g. of nitrogen per day. This included 0.35 g. of nitrogen of unknown nature

(contained as impurities in the dietary ingredients) and 0.46 g. derived from the D-isomers of valine, isoleucine and threonine. The amount of nitrogen in the diet was progressively decreased in successive ten-day periods by first removing the urea from the diet and then excluding part of the glycine. The adequacy of the diet was established by determinations of nitrogen balance over the last six days of each period.

Throughout each experiment the intakes of essential amino acids and calories remained unaltered for each individual. Each subject was given a large excess of calories (54.9 and 54.1 Calories per kilogram of body weight) since earlier work showed that large amounts were necessary for maintenance of positive nitrogen balance in subjects fed amino acid mixtures (W. C. Rose, M. J. Coon, and G. F. Lambert, *see Nutrition Reviews* **13,** *71 (1955)*).

The results obtained from both individuals showed that positive nitrogen balance was obtained with as little as 3.5 g. of nitrogen per day over a ten-day period. This was supplied by the L-amino acids (1.42 g. of nitrogen), by the D-amino acids (0.52 g. of nitrogen), by glycine (1.21 g. of nitrogen), and by unknown impurities (0.35 g. of nitrogen). This is equivalent to a total of only 21.9 g. of protein per day (nitrogen × 6.25), or 18.6 g. when the D-forms of the amino acids are not considered. The authors point out that positive nitrogen balance could probably have been obtained with even lower amounts of nitrogen (from 2.28 to 2.55 g.) if the minimal levels of amino acids had been used (rather than "safe intakes" at twice minimal values).

Several questions arise from examination of the data. Only 2 individuals were studied. One subject gained 5.2 kg. (11.4 pounds) of body weight over the two-month period and the second subject gained 1.8 kg. (3.9 pounds). It is likely that this increased weight includes a small amount of body protein. If so, even lower nitrogen requirements might have been obtained under true maintenance conditions. The question of whether sufficient time was used in each period fully to establish positive nitrogen balance needs further study (D. M. Hegsted, *Nutrition Reviews* **10,** *257 (1952)*; *Ibid.* **13,** *42 (1955)*). Also needed are similar studies made with a greater number of individuals and with different sexes.

The calculated daily protein requirement of 22 g. is considerably less than the value of 1 g. per kilogram of body weight per day recommended by the Food and Nutrition Board (*National Academy of Sciences—National Research Council, Washington, D. C., Publication No. 302 (1953)*). However, limitations in recommending such low values as those obtained by Rose and co-workers have been pointed out by the Food and Nutrition Board (*loc. cit.*). The value is remarkably close to the value of 20 g. of protein per day which was sufficient to maintain positive nitrogen balance in patients on a rice diet (D. M. Watkin, *J. Clin. Invest.* **29,** *851 (1950)*; *Nutrition Reviews* **9,** *255 (1951)*).

Although the data are very limited they are of basic importance and present further evidence that the nitrogen requirement of apparently well adults is lower than was previously thought. Such information should act as a stimulus for further study.

DIETARY SULFUR AND URINE ACIDITY

Some of the earliest observations of the influence of diet on urine acidity were recorded by Claude Bernard, who clearly recognized that a meat diet resulted in an acid urine. It has since been stated that the reaction of the urine depends upon the dietary content of fixed anions and cations. Thus if dietary sodium, potassium and

calcium are in excess of the anions which cannot be metabolized, such as chloride, then the urine will be alkaline. If, however, dietary sulfur and phosphorus, which are generally constituents of proteins, are in excess of the above cations, then the urine will be acid. The only criticism of these views that has been offered is that the food phosphorus usually exists as the phosphate at the time of ingestion and thus must be neutralized by equivalent cations, therefore behaving as an essentially neutral substance.

J. N. Hunt (*Clin. Sci.* **15,** *119 (1956)*) re-investigated this problem by studying normal subjects on a variety of acid and alkaline ash diets. Four subjects were placed on a milk diet for a period of four days. Milk has an alkaline ash and in this experiment each subject received daily an average of 125 milliequivalents (mEq) of cation excess over metabolizable anion. The urine was titrated to pH 7.0 and ammonia was determined to give a measure of total acid. It was found that the subjects excreted urine with a total acidity of 67 mEq daily and the urine sulfate averaged 71 mEq daily. These data demonstrate that it is possible for an individual to excrete an acid urine while receiving an alkaline ash diet and suggest that the acid might originate from dietary sulfur.

To test this hypothesis further, 2 subjects were given large amounts of lettuce to eat. Lettuce is an alkaline ash food with 3.8 mEq per 100 g. and contains almost no protein. Each subject averaged an excess of 160 mEq of alkaline ash. There was no significant change in either the output of total acid or of sulfate, again demonstrating the parallel between sulfate excretion and acid excretion and the lack of influence of the alkaline ash content of the diet on the latter.

Hunt then attempted to reduce urinary sulfate. One subject lived for seven days on a diet essentially without protein, but calorically adequate. By the seventh day, the urine pH was 7.12. Total urine acid had dropped to 22 mEq per day and urine sulfate was 32 mEq per day. It was assumed that this was an alkaline ash diet. Two subjects who took no food of animal origin in their diets were studied. In one, although the dietary alkaline ash averaged 59 mEq daily, the urine was acid seven days out of nine, and the total acid averaged 27 mEq per day, with a urine sulfate of 22 mEq daily. The data for the other patient were essentially similar.

It was subsequently shown that adding methionine to the diet led to a prompt increase in total urinary acid, which could be accounted for by the excretion of 50 to 80 per cent of the administered methionine sulfur. This result was confirmed in several patients.

The author then calculated the percentage of urinary output of acid which can be accounted for by the assumption that the urinary sulfate is equivalent to the amount of sulfur oxidized in the body. When the percentage was less than 100, he made an effort to account for the extra acid. He could derive no support for the assumptions that sulfate was excreted into the intestine or that amino acids and organic acids comprised this moiety. In only 3 of 8 studies was there an appreciable discrepancy between the total acid and sulfate excretion.

Some additional information could be gleaned from these studies. The fluctuations of titratable acid could be closely correlated with the daily dietary intake of phosphorus in the one patient who showed marked fluctuations. It might be expected that a subject receiving phosphate would excrete more titratable acid, and this was realized in these studies.

In summary, it was impossible to demonstrate a relationship between the acid or alkaline ash of the diet and the total acid of the urine. However, total acid of the urine correlated well with the urinary excretion of sulfate.

UNSATURATED FATTY ACIDS AND SERUM CHOLESTEROL LEVELS

In the last few years, studies reported by at least three laboratories have shown that diets containing certain of the more unsaturated vegetable oils as the source of fat are capable of lowering serum cholesterol significantly in human subjects (*Nutrition Reviews* **13,** *8, 44 (1955)*; **14,** *107 (1956)*). Generally, in such studies, formula-type diets have been used rather than diets composed of natural foods.

The study of B. Bronte-Stewart, A. Atonis, L. Eales, and J. F. Brock (*Lancet* **I,** *521 (1956)*) is of related interest.

This investigation took as its point of departure an epidemiologic study made in South Africa (B. Bronte-Stewart, A. Keys, and J. F. Brock, *Lancet* **II,** *1103 (1955)*) which indicated that widely differing interracial incidence of atherosclerosis was associated with a parallel difference in serum cholesterol levels. Furthermore, in each racial group the serum cholesterol levels became higher as the intake of animal fat increased. There was no such correlation with an increase in dietary vegetable fat. With these guiding facts the investigators began a comparison of the effects of various animal and vegetable lipids on the serum cholesterol levels in man, using 5 volunteer Bantu male natives, a Cape Colored male and 2 European male patients as subjects.

The natives originally all had low serum cholesterol values and the Europeans, aged 38 and 42, had relatively high cholesterol levels with clinical evidence of coronary heart disease. The ration for the Europeans included a moderate intake of animal fat (50 g. daily) while the basic diet for the 6 non-European volunteers consisted of maize-meal products and white bread supplemented with sugar and casein. The latter ration contained no cholesterol and not more than 3 per cent of the total calories was derived from fat. To these rations various fats and oils were added, usually one at a time, to increase the proportion of calories derived from fat to 36 per cent, 45 per cent and even up to 60 to 70 per cent.

Many different fats were tested. The results were evaluated in terms of changes in the serum cholesterol and, in some instances, beta-lipoprotein concentrations, which were determined by paper electrophoresis every other day. Usually the changes in concentrations of these two blood lipid fractions paralleled each other.

Although it is difficult to summarize briefly the results of all of the dietary combinations tested, the following findings are representative: Adding animal fat in the form of beef tallow or butter to the Bantu basic diet resulted in a prompt rise in serum cholesterol whereas equivalent quantities of olive oil had no such effect. In the Bantu a hydrogenated nut fat produced a rise in serum cholesterol whereas a comparable natural nut oil had no such effect. In the Europeans 100 g. of sunflower seed oil added to a ration which already contained 100 g. of beef fat caused a definite and sustained drop in serum cholesterol. A similar effect was shown when the sunflower seed oil was combined with other animal fats. Highly unsaturated marine oils such as pilchard oil produced an even more marked depressant effect on serum cholesterol when combined with dietary animal fats. Since the results up to that point supported the idea that dietary unsaturated fats lower serum cholesterol while the saturated fats cause a rise in serum cholesterol, sunflower seed oil was separated into saturated and unsaturated fractions. Feeding of these fractions with and without added animal fat or cholesterol supported the basic premise that the serum cholesterol and beta-lipoprotein levels were readily controlled by altering the concentrations of saturated or unsaturated lipids in the diet. In general, 27 per cent of the ingested fat as linoleate is suggested as

a critical level to prevent a rise in the serum cholesterol when abundant animal fat and cholesterol are being ingested.

On the basis of their data Bronte-Stewart and co-workers conclude that the fatty acids are responsible for the effects observed and not the sitosterol or vitamin E which might have been present in the diets. The marine oils tested, for example, contained no sitosterol and only a little alpha-tocopherol, yet they produced reduction in the serum cholesterol levels comparable to the unsaturated vegetable oils. Dietary protein, which was varied over a wide range, also seemed to have little effect on the serum cholesterol level in these experiments.

From preliminary results on one patient fed a formula diet in which a synthetic triglyceride was the source of dietary fat and in which the twenty-four-hour intake of linoleic acid was approximately 2 g., L. W. Kinsell, R. W. Friskey, G. D. Michaels, and F. R. Brown (*Am. J. Clin. Nutrition* **4,** *285 (1956)*) conclude that their observations are "compatible with the concept that the plasma cholesterol and phospholipid levels are a function of the relative or absolute intake of essential fatty acids."

While the results reported by Bronte-Stewart and colleagues are remarkably uniform and clear-cut, it should be pointed out that only 8 subjects were involved and the feeding periods of the various oils were quite short, ranging from only 5 to 12 days. It is known that spontaneous cholesterol variations are appreciable. It is conceivable that marked changes in the nature and amount of fat in the diet might promptly affect cholesterol absorption and hence its level in the serum. But it would also be expected that in a short time the body would compensate and the level of cholesterol in the serum return to the control level. The data in this paper from South Africa would be far more impressive if the cholesterol values of the subjects had been followed at weekly intervals for five to six weeks prior to the oil feeding periods and if the feeding periods had been of five to six weeks duration rather than only one to two weeks.

That large concentrations of unsaturated vegetable oils included in formula feedings result in a decrease of serum cholesterol in a majority of individuals seems established. However it has not yet been established that a similar result, except perhaps for a transient period, is obtained if such fats are included in an ordinary diet to the extent that they provide 30 to 40 per cent of the total caloric intake, a level that might be compatible with practicality.

RIBOFLAVIN BLOOD LEVELS IN MAN

The value of blood riboflavin levels as a measure of nutritive status with respect to this vitamin has been investigated in some extensive studies conducted at the Elgin State Hospital, Elgin, Illinois (O. A. Bessey, M. K. Horwitt, and R. H. Love, *J. Nutrition* **58,** *367 (1956)*). Data were obtained on a total of 47 subjects in two studies.

In the first study, 10 subjects received a diet adequate in all known nutrients, but containing only 0.55 mg. of riboflavin per person per day for a sixteen-month period. At this time, when all subjects exhibited clinical manifestations of ariboflavinosis, the blood was analyzed for riboflavin. A second group of 6 subjects received the same diet with riboflavin increased to 2.55 mg. for fourteen months and 3.55 mg. for the final two months.

All blood riboflavin analyses were done in quadruplicate on postabsorptive specimens. Total riboflavin in red blood cells averaged 11.7 micrograms per 100 ml. of blood (range, 10.0 to 13.1) for the subjects on low ribo-

flavin intake contrasted with an average of 22.3 micrograms per 100 ml. (range, 20.2 to 27.6) for the subjects on the higher riboflavin intake. The total riboflavin in white blood cells averaged 192 micrograms per 100 ml. of blood for the low-riboflavin group, and 212 micrograms per cent for the adequate-riboflavin group, with considerable overlapping between the two groups. Total plasma riboflavin likewise varied considerably in subjects within groups, and averaged 2.36 micrograms per 100 ml. for the low-riboflavin group, and 3.03 micrograms per cent for the adequate-riboflavin group. Partitioning of the plasma riboflavin revealed that the free vitamin plus the flavin mononucleotide averaged 0.28 and 0.71 for the subjects on the low and adequate riboflavin intakes respectively. The flavin adenine dinucleotide values were nearly the same in the plasma of the two groups of subjects.

From the data, it was evident that the total riboflavin in red blood cells was strikingly and consistently lower in the riboflavin-restricted group as compared with that of subjects on an adequate riboflavin intake.

The second study was primarily designed to investigate niacin requirements. Four groups of 7 to 8 subjects each received a basal diet varying in tryptophan and niacin, and with riboflavin at the following levels: group 1, 0.5 mg. per day; groups 2, 3 and 4, 2.4 mg. Data were also obtained on a fifth group, which received the regular hospital diet (1.6 mg. of riboflavin per day). Subjects on the restricted riboflavin intake showed a gradually decreased total red blood cell riboflavin from an initial level of 21.1 micrograms per 100 ml. of blood to a level of 11.9 micrograms per cent after nine months on the diet. The subjects receiving 1.6 or 2.4 mg. of riboflavin daily remained very nearly at the initial levels of 20 micrograms per cent throughout the experiment, an indication that they were in good riboflavin nutrition.

Following the nine-month experimental period, the subjects of group 1 were divided into 2 groups, one of which received a total of 1.3 mg. of riboflavin daily for seventy-one days and 2.4 mg. for a subsequent fourteen-day period. The other subgroup of 4 subjects received 2.4 mg. of riboflavin daily for the eighty-five-day period. Riboflavin realimentation appeared to be complete in all subjects of the latter group after the eighty-five-day period, whereas the subgroup receiving only 1.3 mg. of riboflavin daily appeared to respond somewhat more slowly.

From the data, it appears that red blood cell riboflavin content can serve as a reliable measure of riboflavin intake or dietary adequacy in man. Bessey and associates conclude that 20 micrograms of riboflavin in red blood cells per 100 ml. of blood would indicate an adequate intake. This concentration occurs with intakes of 1.5 to 2.5 mg. per day. Intake levels as high as 10 mg. per day resulted in only a transient rise over the 20 microgram per cent level, so this appears to represent the saturation level for red blood cells. They further conclude that red blood cell levels below 15 micrograms per 100 ml. of blood would indicate suboptimum riboflavin intake. With one exception, all subjects in the study whose red cell riboflavin concentration was below this level developed clinical symptoms of riboflavin deficiency.

In an earlier report these workers summarized the urinary excretion of riboflavin in the subjects used in these studies (*Nutrition Reviews* **9,** *49 (1951)*). They noted that levels of excretion below 50 micrograms daily were indicative of prolonged suboptimum riboflavin intake. The urinary excretion pattern, however, would probably be less reliable than red blood cell concentration, since small amounts of riboflavin-rich food were demonstrated to increase, temporarily and misleadingly, the urinary excretion rate, but had very little if any influence on the red blood cell level.

ANALYSIS OF BODY COMPOSITION

The variety of methods available for assessment of body composition includes biochemical methods (analyses of the whole body for fat, total body water, protein, total ash), X-rays of the skeleton, soft tissue roentgenograms (H. C. Stuart, P. Hill, and C. Shaw, *Monog. Soc. Research Child Develop., Vol. 5, No. 3, Serial No. 26, Washington, D. C. (1940)*), body density measurements and external body measurements (skinfold thickness and visual assessment).

Each of these technics by itself has such considerable limitations, especially when applied to human subjects, that a synthesis of methods including the anatomic, chemical and histochemical approaches seems desirable. It is hoped that once the relationships, errors and reliabilities of the various methods have been established, certain simple and conveniently applied measurements may suffice for many experimental procedures.

J. Brozek (*Ann. N. Y. Acad. Sci.* **63,** *491 (1955)*) has attempted such a synthesis, focusing particular attention on the problem of determining the total amount of body fat by anthropologic measurement. The methods used consisted for the most part of measurements of skinfolds correlated with densitometric analysis. However, whenever possible, biochemical and biophysical measurements were also considered.

The skinfold measurement is one of the most convenient to apply to human subjects but suffers from two major limitations: (1) the method yields no reliable information on internal fat deposits; (2) individuals have been found to have different surface lipid deposition patterns. Actually, it has been considered that such differences in site of deposition might lead to additional and useful information in nutritional studies. Skinfold measurements and soft tissue roentgenograms allow a numerical portrayal of the distribution pattern. By relating the departure of an individual value for each site (Xi) from the group means ($\overline{X}$) to the standard deviation $Z = (Xi - \overline{X}/S.D.)$, one can arrive at a relative fat pattern. In most cases, however, it must be admitted that these variations lead merely to analytic error unless carefully considered and interpreted. S. M. Garn (*Human Biol.* **26,** *59 (1954)*) has found that the fat pattern changes little in the course of weight loss associated with maintenance for twenty-four days on survival rations. Even an 11 per cent drop in body weight, accompanied by considerable loss of fat, elicited no sharp alterations in relative fat distribution.

The objections to skinfold measurements because of individual differences have been somewhat lessened by the studies of J. Brozek and A. Keys (*Brit. J. Nutrition* **5,** *194 (1951)*) and of L. R. Pascale, M. I. Grossman, and H. S. Sloane (*Army Med. Nutrition Lab. Report No. 162, Denver, Colorado (1955)*). These workers have found that certain skinfolds correlate better than others with over-all leanness and fatness. Folds of the abdomen, chest and arm seem to be the most indicative of general body composition. In two age groups (20 and 49 years) the skinfold at the back of the upper arm was found to have the largest weight in prediction equations.

At the Army Medical Nutrition Laboratories, Denver, Colorado, skinfold measurements were compared with body densities subsequent to development of the following prediction equation:

$$D = 1.08012 - 0.0007123\,S - 0.0004834\,C - 0.0005513\,A$$

where D = the predicted body density; S = the skinfold thickness in mm. measured at the "side" (on the chest in the midaxillary line at the level of the xyphoid process); C = "chest" (in the juxta-nipple position) and A = "arm" (on the dorsum of the arm at the midpoint between the tip of the acromion and the tip of the olecranon).

However, these data were obtained on young men (mean age 22.1 years), and it was necessary to determine if the equation would hold for older individuals. Accordingly, Brozek compared two groups of men, mean age 20.3 and 44 years, respectively. As expected, the mean skinfolds of the older group were thicker than were those of the younger men, and the densities were lower. Using the prediction equation derived for the younger men, however, Brozek obtained 1.0547 as the density predicted for the older men as compared to the actual mean value of 1.0489. The corresponding statistically reliable fat values were 16.0 and 19.2 per cent of body weight. Obviously the internal fat deposits of the older men were greater than those of the younger men, and thus the skinfold test was less reliable with advancing age.

Brozek points out that relative body weight is a particularly poor criterion of fat deposition, especially when employed for groups containing individuals of greatly varying ages. In two groups of men studied (mean ages 20 and 52 years) there was about a 10 per cent difference in body fat between the very light and very heavy individuals. In the older men, approximately another 10 per cent of fat was added, reflecting the age changes in body composition.

Common anthropologic classifications include endomorphic (round body), mesomorphic (square body) and ectomorphic (narrow body) types. A. Keys and J. Brozek (*Physiol. Rev.* **33,** *245 (1953)*) found that endomorphic ratings (1 through 7), from examination of photographs, correlated surprisingly well with densitometric estimates of the fat content of human subjects. Brozek is of the opinion that this method of assessment could be improved even further by careful correlation of densitometry, skinfold measurements and soft tissue roentgenograms. The improved system should focus concisely and directly on the variable aspects of body build.

As an example of the benefits of integration of various approaches to body composition, Brozek quotes recent work (J. Brozek and F. Grande, *Human Biol.* **27,** *22 (1955)*) which concerns the partitioning of basal oxygen consumption among the various organs and tissues of the body. Direct determination of oxygen consumption from arterial and venous vessels, calculation of blood flow rates and estimates of the per cent of various body tissues in the whole body permitted a satisfactory partitioning of total oxygen consumption data. It was concluded that the adipose tissue in the normal human being consumes approximately 15 per cent of the total oxygen utilized by the body.

POTASSIUM DEPLETION AND KIDNEY FUNCTION

Interest in the effects of potassium upon kidney function has burgeoned in the past few years. In animals, subtle defects in the handling of water loads have been elucidated (*Nutrition Reviews* **13,** *148 (1955)*). Experimental potassium depletion has become feasible in man and electrolyte balance has been studied during its development (*Ibid.* **11,** *270 (1953)*). The two studies discussed in the preceding citation demonstrated sodium retention during induction of potassium deficiency. P. Fourman (*Clin. Sci.* **13,** *93 (1954)*) noted the appearance of pulmonary edema during experimental potassium depletion in man. This observation prompted Fourman and G. R. Hervey (*Ibid.* **14,** *75 (1955)*) to study sodium balance carefully during potassium depletion.

The subject was a normal male who was placed on a calorically adequate diet containing 45 milliequivalents (mEq) of potassium and 138 mEq of sodium. Severe potassium depletion was induced by the administration of an ammonium cycle cation

exchange resin for six days. During the entire period there was a loss of 620 mEq of potassium, with 470 of these lost during the period of resin administration. This potassium loss was accompanied by anorexia, asthenia and a drop in serum potassium to 3.3 mEq per liter. There was a 2 kg. weight loss without an accompanying negative nitrogen balance.

Sodium chloride was then given, 256 mEq daily for six days. During this period there was a weight gain of 3.4 kg. due to a positive sodium balance of 689 mEq with consequent retention of water. At this point, 99 mEq of potassium citrate were added to the intake. There was a potassium retention of 435 mEq of potassium and a urinary loss of 453 mEq of sodium. The serum potassium rose to 4.0 mEq per liter.

It was calculated that of the retained sodium, 120 mEq entered the intracellular compartment and 440 mEq remained extracellular, accounting for the observed weight gain. This expansion of extracellular fluid volume was accompanied by a correspondingly decreased hematocrit. There was an apparent net gain of sodium throughout the twenty-four days of the study, probably due to insensible loss.

The retained potassium of the last experimental period entered the cells. The concurrent salt loss was due to 100 mEq of sodium leaving intracellular fluid and 350 mEq leaving extracellular fluid.

In a subsequent study on the same subject maintained on a normal diet, the same salt load resulted in only 272 mEq of sodium retained. Thus it was confirmed that potassium depletion favored a retention of sodium and prevented the efficient functioning of the mechanisms for salt diuresis. The possible role of potassium depletion in activating the secretion of aldosterone (*Nutrition Reviews* **13,** *281* (*1955*)) may be pertinent in this regard.

An incidental finding was that water diuresis remained normal or was possibly enhanced during the period of potassium depletion. This is reminiscent of some studies reported by W. B. Schwartz and A. S. Relman (*J. Clin. Invest.* **32,** *258* (*1953*)) on 2 patients with chronic potassium depletion due to overuse of laxatives. There were depressions in glomerular filtration rate and tubular function which were corrected by potassium repletion. A fixed specific gravity was noted in one patient.

The recent observations that a pitressin-resistant polyuria (excess excretion of urine) in primary aldosteronism is accompanied by hypokalemia (low blood levels of potassium) add another set of observations relating potassium depletion to abnormalities of renal function. From the evidence quoted here, chronic potassium depletion may be seen to lead to an inability to handle salt loads, as well as to interference with renal tubular function as it concerns water conservation. As discussed above, these effects need not necessarily result from interference with renal mechanisms.

INDUCED CHANGES IN BLOOD GLUCOSE AND FOOD INTAKE

Within the last two decades it has been demonstrated that there is an area in the hypothalamus specifically concerned with the regulation of food intake. When the medial portions of this area are experimentally damaged food intake increases out of proportion to energy needs and the experimental animal becomes grossly obese. When lesions are placed in the lateral portions the animal ceases to eat and starves to death unless forcibly fed.

Some investigators have interpreted these results as indicating that there are at least two discrete "centers" in the hypothalamus having to do with energy balance, one a "feeding center" which is directly concerned

with feeding behavior and the other a "regulatory center" which governs the activity of the feeding center.

The knowledge that there exists in the hypothalamus a center concerned with regulation of energy balance has stimulated attempts to elucidate the "feedback" mechanism whereby energy intake is presumably adapted to energy requirements. The various theories and hypotheses developed to explain the manner in which the hypothalamus obtains information about available energy have been discussed in reviews by J. Mayer (*Physiol. Rev.* **33,** *472 (1953)*), J. R. Brobeck (*Nutrition Symposium Series, No. 6, p. 36, New York (1953)*) and M. I. Grossman (*Ann. N. Y. Acad. Sci.* **63,** *76 (1955)*).

One of the provocative and heuristic concepts of food intake regulation has been the "glucostatic theory" proposed by Mayer and his associates. According to this theory, the available carbohydrate supply of the body exerts a regulatory influence on food intake via hypothalamic "glucoreceptors"; the urge to eat increases as carbohydrate stores diminish. The hypothalamus cannot obtain direct information concerning the status of the carbohydrate stores; however, the rate of carbohydrate utilization by the body as a whole tends to be directly proportional to the supply of this nutrient (J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," *Vol. 1, Interpretations, 2nd edition, p. 35, Williams and Wilkins, Baltimore (1946)*). Accordingly, measurement of the rate at which blood glucose is utilized provides indirect information about available carbohydrate supply.

In a preliminary study on human subjects, T. B. Van Itallie, R. Beaudoin, and J. Mayer (*J. Clin. Nutrition* **1,** *208 (1953)*) attempted to correlate peripheral capillary-venous glucose differences (as convenient indicators of extrahepatic glucose utilization rates) with naturally occurring states of hunger and satiety. Glucose content of capillary blood samples does not differ significantly from that in arterial samples. They found that when the arteriovenous (A-V) glucose difference remained small, hunger tended to be either actively present or imminent. In contrast, hunger was rarely present when large peripheral A-V glucose differences were measured. By using glucose A-V differences rather than venous glucose values alone as an index of available carbohydrate, the authors were able to account for the fact that in uncontrolled diabetes mellitus marked hunger frequently occurs in the presence of high blood glucose levels. However, in such instances, the peripheral A-V difference remains small.

Subsequently, A. J. Stunkard and H. G. Wolff (*Fed. Proc.* **13,** *147 (1954)*) injected glucose intravenously into volunteer subjects whose gastric contractions were being simultaneously recorded. They found that contractions tended to be abolished in the subjects who displayed larger peripheral A-V glucose differences following glucose injection whereas in subjects with relatively small A-V glucose differences after glucose injection (for example, diabetic patients from whom insulin had been withheld) inhibition of gastric motility was not apparent. Stunkard, T. B. Van Itallie, and B. B. Reis (*Proc. Soc. Exp. Biol. Med.* **89,** *258 (1955)*) showed that by injecting glucagon (*Nutrition Reviews* **13,** *323 (1955)*) (the hyperglycemic glycogenolytic factor in pancreatic extracts) it was possible repeatedly to abolish gastric hunger contractions in man. Abolition of gastric hunger contractions at this time was associated with increased peripheral A-V glucose differences.

J. H. Fryer, N. S. Moore, H. H. Williams, and C. M. Young (*J. Lab. Clin. Med.* **45,** *684 (1955)*; *Nutrition Reviews* **13,** *259, 299 (1955)*) measured peripheral arteriovenous glucose differences in 12 overweight subjects fed reducing diets in which the proportions of carbohydrate, fat, or protein were varied. They found that there was a marked reduction in the satiety value of the diets which contained the lower protein com-

ponents and which were associated with the lowest rates of peripheral glucose uptake. Accordingly, these investigators suggested, as an alternative to the glucostatic theory, that some balance between metabolites within the metabolic pool (rather than glucose alone) might provide the humoral mechanism controlling appetite through the mediation of the hypothalamic centers.

However, the studies of Fryer *et al.* were done on overweight subjects in negative caloric and possibly negative nitrogen balance; therefore, it is not certain whether the results can be applied to nonobese individuals in caloric and nitrogen equilibrium. Moreover, V. P. Dole and associates (*Am. J. Clin. Nutrition* **2,** *381 (1954)*; *Nutrition Reviews* **13,** *259 (1955)*) have reported that diets low in protein also have an appetite-suppressing effect and are useful in the treatment of certain forms of obesity. These apparently contradictory findings suggest that the protein intake may affect satiety by more than one mechanism, but the nature of the mechanisms involved remains obscure.

In an attempt to test the glucostatic theory, L. M. Bernstein and M. I. Grossman (*J. Clin. Invest.* **35,** *627 (1956)*) studied the effect of induced changes in blood glucose level on the quantity of food eaten under experimental conditions. They also studied the effect of induced hyperglycemia on the desire to eat, as evaluated subjectively.

Nine young adult males served as test subjects. Food consumption tests were performed every other day, three times weekly for three weeks for a total of nine tests on each subject. On the days of the tests, the subjects consumed completely a fixed breakfast of 580 Calories served at 7:30 a.m. At 11:30 a.m. the test treatments were given and at noon the subjects were offered the test meal which they were permitted to consume ad libitum.

The test treatments used were 200 ml. of 25 per cent glucose in water administered by fine polyethylene stomach tube, 200 ml. of 10 per cent glucose in water given for a period of fifteen minutes by intravenous infusion, 200 ml. of 0.9 per cent sodium chloride solution containing 10 mg. of DL-amphetamine given by fine polyethylene stomach tube, orally administered 0.9 per cent sodium chloride solution, and intravenously administered 0.9 per cent sodium chloride solution. Capillary (arterial) and venous blood samples were obtained immediately before the test treatment, thirty minutes later (immediately before the test meal), and at the end of the test meal.

The test meal offered consisted of an appetizing selection of foods, such as tenderloin steak, baked potatoes, corn, salad and vanilla ice cream, available in unlimited quantities. The subjects were told that they could consume as little or as much of each food as they wished and that the purpose of the study was to evaluate the effect of meals on the blood glucose. Apparently, no attempt was made to find out how these instructions were construed by the subjects. Instructions of this sort are so frequently inverted in the minds of experimental subjects that many psychologists prefer to give totally irrelevant instructions when their motive is to distract the subjects from the true purpose of the experiment.

Peripheral arteriovenous glucose differences after glucose was administered intragastrically and intravenously were significantly higher than those obtaining prior to the test treatments. However, despite the increased glucose uptake following glucose administration, the test treatments, including amphetamine administration, were followed by no significant decrease in intake of food at the time of the ad libitum meal, nor was there any effect of any of the procedures on subjective "appetite."

The authors maintain that, since significantly increased glucose utilization rates are not, in their experiments, associated with an appreciable lowering of food intake,

doubt is cast upon the validity of the glucostatic theory.

In interpreting the results obtained by Bernstein and Grossman, it is worth noting their finding that DL-amphetamine did not significantly decrease food intake under their experimental conditions. This finding is contrary to the well-established fact that amphetamine, when it is administered under appropriate conditions, has an inhibitory effect on food intake both in experimental animals and human subjects (S. C. Harris, *Ann. N. Y. Acad. Sci.* **63,** *121* (*1955*)). The failure of amphetamine to inhibit food intake in the experiments of Bernstein and Grossman suggests strongly that something was wrong with their experimental design. It would appear that the methods used by these authors to assess the effect of various test procedures upon appetite and food intake were not suitable for obtaining clear-cut answers to the questions under consideration.

When the experimental design employed by Bernstein and Grossman is carefully examined, several important flaws come to light. First, it must be borne in mind that the subjects under study may have been raised in homes where the desirability of finishing what was on the plate was firmly and repeatedly emphasized. Such habits developed in early life are strongly ingrained and, within rather wide limits, most adults will consume what is placed before them regardless of the state of their appetite at the time. When the "test" meal to be eaten consists of palatable foods, such as steak, corn and vanilla ice cream, it would seem that positive motives (pleasure) for finishing the meal would combine with negative motives (guilt) to prevent the subject from leaving food on his plate.

Clearly, the mechanisms which act to stop eating are not very strong or rigid under natural circumstances. It is a common observation that if an extra amount of appetizing food is available during a meal, it will frequently be consumed even though its calories are not needed to maintain energy balance. Accordingly, the experimental design of Bernstein and Grossman is questionable both in respect to details and, in a broader sense, in its assumptions. It would certainly have been far better to have had the subjects consume a formula-type test meal without knowledge of the amount consumed or its food value. However, since the brake on overeating is apparently not a strong one, it may be questioned whether isolated experiments on well nourished young subjects of the kind performed by Bernstein and Grossman will provide satisfactory results regardless of how far the procedure is refined. It may ultimately be more rewarding to perform the kind of experiment in which the effect of increased energy expenditure is correlated with changes in carbohydrate metabolism and food intake. The need to satisfy increased energy requirements by increasing food intake seems to be biologically more crucial than the need to avoid "overweight."

Bernstein and Grossman have initiated experiments in an area of energy balance physiology that has needed careful exploration. Their studies should provide a point of departure for further work on this phase of the subject. Unfortunately, the results reported by Bernstein and Grossman are inconclusive and the crucial experiments to test the validity of the glucostatic theory remain to be performed.

It should be emphasized that the correlation of states of hunger and satiety with peripheral arteriovenous glucose differences is not at the foundation of the glucostatic theory. Use of peripheral A-V differences as indicators of the rate of glucose assimilation represents an improvement over the measurement of venous glucose levels alone. Yet it remains to be demonstrated that the rate of extraction of glucose by the tissues of the forearm is representative of glucose utilization by the body as a whole. Thus, it seems desirable not to focus undue attention upon

the A-V difference as having some mysterious intrinsic significance but to look beyond this to the postulated relationship between the carbohydrate available to hypothalamic receptors and the urge to eat. Unless the glucostatic theory is thought of in terms of this relationship, it is likely that experiments designed to test the theory (which is not yet established) will continue to be inconclusive.

METHIONINE TOXICITY IN LIVER DISEASE

The role of the sulfur-containing amino acids in preventing hepatic necrosis in animals is well established (*Nutrition Reviews* **12,** *312 (1954)*). For this reason, as well as because of its ability to serve as a source of methyl groups, methionine has been administered to patients with cirrhosis, but with variable success. In several instances, methionine supplementation was noted to precipitate neurologic changes indistinguishable from those seen in impending hepatic coma. These observations and the recently analyzed effects of a high-protein diet or ammonium salts in precipitating hepatic coma (*Ibid.* **14,** *74 (1956)*) suggested a study of the mechanism of action of methionine in aggravating hepatic coma.

E. A. Phear, B. Ruebner, S. Sherlock, and W. H. J. Summerskill (*Clin. Sci.* **15,** *93 (1956)*) have studied the effects of methionine in 28 patients with liver disease. Nine of these patients had previously experienced episodes of impending hepatic coma. The degree of neurologic disorder was graded and assessed by 2 observers during methionine administration. Methionine was usually given in enteric-coated tablets containing 250 mg. of D,L-methionine in a daily dose averaging 10 g. Intravenously administered methionine was tested on four occasions, 6 g. being given over a thirty-minute period.

Of the 9 patients who had pre-existing neurologic complications associated with liver disease, 7 showed neurologic deterioration when methionine was given. The pattern was the same as that noted during spontaneous deterioration. It began one to four days after commencement of methionine administration and after total doses of 11 to 46 g. Four of these 7 patients received methionine intravenously, but only one showed clinical deterioration.

Seven patients with portal cirrhosis who had never exhibited neurologic complications tolerated 50 to 100 g. of methionine without incident.

Control blood ammonium levels were raised in the group who showed deterioration upon methionine administration. However, after methionine was given there was an inconstant change in blood ammonium levels in both groups, making it difficult to implicate ammonium poisoning as the cause of the neurologic symptoms. The one patient who responded adversely to intravenous methionine displayed a significant rise in blood ammonium. The rise in blood methionine levels was comparable in both groups. There was no change in serum bilirubin or serum bicarbonate between the two groups.

Five of the patients who had responded adversely to methionine administration were given 2.0 g. of chlortetracycline on two days before and during another period of methionine administration. These patients were completely protected by the chlortetracycline but their blood ammonium levels showed no change. Bacteriologic investigations of intestinal flora before chlortetracycline administration disclosed no significant differences between the groups with and without neurologic complications.

These studies strongly suggest that methionine supplementation should be abandoned in severe liver disease. The reasons for its toxicity have not been elucidated, but several mechanisms have been in-

vestigated. Ammonium derived from methionine seems an unlikely cause of the neurologic sequelae produced by the methionine. However, other breakdown products of methionine may be responsible for these changes.

The protection afforded these patients by chlortetracycline suggests that bacterial action on methionine leads to production of toxic substances. It is suggested by the authors that studies of the mercaptans and amines derived from methionine may prove fruitful in elucidating the cause of this action of methionine in liver disease.

UTILIZATION OF MATERNAL IRON FOR BLOOD FORMATION IN INFANCY

During hematologic studies of pregnant women, the opportunity was presented to study the utilization of transplacental iron (C. A. Smith and co-workers, *J. Clin. Invest.* **34,** *1391* (*1955*)). Some of the facets of iron metabolism in infancy have been summarized (*Nutrition Reviews* **11,** *163* (*1953*)), but there are numerous areas unexplored. H. W. Josephs (*Medicine* **32,** *125* (*1954*)) examined balance data for iron in infants and noted that under 3 months of age there was net iron equilibrium with a daily intake of iron of about 0.15 mg. per kilogram of body weight. Balance data, however, do not permit the differentiation of nonutilization of ingested iron from absorption and excretion. Furthermore, a variable and previously unmeasurable iron "store" could contribute to the increasing total hemoglobin seen during the first months of life.

In the studies of Smith and co-workers radioactive iron (Fe^{55}) was administered to 11 pregnant women in the form of donor red cells in transfusions of 100 ml. of whole blood on four to seven occasions. The first transfusion was given 230 days antepartum, the last ten days before delivery. Breakdown of these cells released radioactive iron which labeled the fetal iron pools. At varying times after delivery the specific activity of the hemoglobin iron (ratio of radioactive iron to hemoglobin iron) was determined and related to the specific activity in the cord blood, which was taken as 100 per cent. Thus, a decrease in iron specific activity would imply the use of iron coming from a nonlabeled pool, presumably from the diet. The assumption, stated by the authors, is that all the fetal iron pools are equally labeled. This premise is necessary in order to draw any conclusions from changes in specific activity.

When specific activity was plotted against time, it was seen that there was little change until about 100 days, suggesting that there was no appreciable use of food iron for hemoglobin formation. The total circulating hemoglobin was estimated using a calculated blood volume based on physical measurement of each infant at the time of sampling. With this datum it was then possible to calculate the amount of circulating hemoglobin derived from transplacental iron. This was found to increase slightly at 60 days before there was evidence of utilization of dietary iron. Thus "stores" of transplacental iron must have contributed to new hemoglobin synthesis.

As the amount of transplacental iron emerging from "stores" varied among the infants, the authors tried to determine if the total hemoglobin iron reflected the iron "stores." However, those infants who displayed the largest additions of stored iron tended to have lower total hemoglobins at birth, implying a reciprocal relationship between total circulating hemoglobin and iron "stores."

The importance of the iron "stores" was suggested by the protocols of 2 infants. A premature infant, born of a mother whose dietary intake was poor, had a normal total hemoglobin for his weight. During the first thirty months of life, although the total

hemoglobin increased, none of this increase was due to transplacental iron, suggesting inadequate "stores." Another infant, full-term, whose mother had been receiving an adequate diet, had a slightly low total hemoglobin at birth. He received medicinal iron during his first year but none of this contributed to the hemoglobin iron during the first seven months. During this time, however, 100 mg. of transplacental iron was used for hemoglobin synthesis from iron "stores."

The authors have shown that infants utilize little dietary iron until four months after birth. At 2 years, 90 per cent of the transplacental iron was still being used in hemoglobin. Transplacental iron in non-hemoglobin "stores" may be utilized in hemoglobin synthesis.

IDIOPATHIC HYPERCALCEMIA

In 1952 R. C. Lightwood (*Arch. Dis. Child.* **27,** *302* (*1952*)) described a syndrome in infants, idiopathic hypercalcemia (high blood calcium levels of unknown cause), in which there was failure to thrive. Later it was suggested that there were two varieties of this disorder, one being a severe type associated with mental retardation and having a poor prognosis, and the other being benign, not associated with congenital defects and having a shorter course with usually a better prognosis. J. H. Hutchison (*Brit. Med. J.* **2,** *339* (*1955*)), in discussing this syndrome, noted that these infants showed no evidence of any known disorder which would produce a hypercalcemia, such as hyperparathyroidism, hypervitaminosis D, excess ingestion of alkali, or prolonged immobilization. These patients have, in addition to hypercalcemia, increased levels of urea nitrogen and cholesterol in the blood. Clinically they show a striking degree of apathy.

It has been suggested that this disorder is due to an abnormal sensitivity to vitamin D, coupled with intakes in excess of the actual requirements (K. G. Lowe, J. L. Henderson, W. W. Park, and D. A. McGreal, *Lancet* **II,** *101* (*1954*); R. D. Creery and D. W. Neill, *Ibid.* **II,** *110* (*1954*)). In fact, R. E. B. Carter, C. E. Dent, D. I. Fowler, and C. M. Harper (*Arch. Dis. Child.* **30,** *399* (*1955*)) administered 10,000 I.U. of vitamin D per day for eleven days as calciferol to an infant with idiopathic hypercalcemia, and from the coincidental clinical deterioration concluded that there was an abnormal sensitivity to the vitamin in this individual. On the other hand, Creery and Neill reported that 2 patients they studied were on a dietary intake of only 700 I.U. of vitamin D per day and received no supplementary sources. One of the children with an active case of the syndrome was given 2000 I.U. of calciferol per day for three and a half weeks with steady improvement in clinical state. There was only a transient increase in his serum calcium levels.

More recently J. O. Forfar, C. L. Balf, G. M. Maxwell, and S. L. Tompsett (*Lancet* **I,** *981* (*1956*)) have reported studies with 6 infants with the benign type of idiopathic hypercalcemia. Calcium and phosphorus balance studies were undertaken, and it was found that on normal diets (0.65 to 1.5 g. of calcium per day) the 3 children with active disease retained 31 to 69 per cent of the ingested calcium. Two infants, one with inactive disease and the other in the early recovery stage, each retained 16 per cent of the ingested calcium. Various factors which influence the absorption of calcium in healthy individuals plus the additional complicating influences in these infants such as anorexia, vomiting and constipation make the rigid interpretation of this small series of studies difficult, yet it is believed that the data indicate an increased absorption of

calcium from the diet. This assumption is supported by observations of increased urinary calcium losses, elevated serum calcium levels and possibly increased calcium deposition in the bone. H. G. Morgan, R. G. Mitchell, J. M. Stowers, and J. Thomson (*Ibid.* **I,** *925 (1956)*), using balance technics, have also reported that there is an increased absorption of calcium from the diet by infants with this syndrome. Three of the patients studied by Forfar *et al.* received during one study period their normal diet plus 100,000 I.U. of calciferol per day. The patient with active disease who had been absorbing 48 per cent of his calcium intake now absorbed 73 per cent. The infant with inactive disease increased his calcium uptake from 16 per cent to 42 per cent after receiving the excess vitamin D. The addition of calciferol did not alter the clinical condition nor was there an increased excretion of calcium above that which would be expected in a normal infant receiving the same dose of calciferol.

The hypercalcemia which occurs in this syndrome could be reduced either by giving a low calcium diet or, more rapidly, by giving the steroid hormones, prednisolone or cortisone. It has been suggested that the hormones act by increasing the fecal excretion of calcium. In other words, their effect is that of an antivitamin D. The balance data of both Morgan *et al.* and Forfar and collaborators have shown that patients with idiopathic hypercalcemia have increased absorption of dietary phosphorus and this too can be reduced by the use of cortisone.

H. E. Harrison and H. C. Harrison (*Yale J. Biol. Med.* **24,** *273 (1952)*) reported that the mean serum citrate level in infants up to 2 years of age is 2.5 mg. per cent, with a range of 2.0 to 3.4 mg. Forfar *et al.* found the serum citrate level in one child with an active case of idiopathic hypercalcemia to be 0.7 mg. per cent, and during recovery the citrate level increased to 1.8, 2.4, 2.5 and 1.8 mg. per cent. In hypervitaminosis D Harrison (*Pediatrics* **14,** *285 (1954)*) found serum citrate levels up to 6 mg. per cent. Thus the infant with active idiopathic hypercalcemia had a low plasma citrate level which approached normal as he was recovering. This is an important observation and the measurement should be repeated on more infants having this disorder. The urinary output of citrate was low during the active phase of the disease and approached normal during recovery. In hypervitaminosis D there is an increased amount of citrate found in the urine. Thus, in regard to citrate metabolism patients with idiopathic hypercalcemia do not react as if they were responding to excessive vitamin D activity.

In many of these cases hypercholesterolemia has been noted along with hypercalcemia. Forfar *et al.*, from a study of 4 infants with idiopathic hypercalcemia, obtained 22 pairs of coincidentally drawn blood samples on which serum calcium and plasma cholesterol determinations were made. By gathering similar data from the literature they found that Lowe *et al.* (*loc. cit.*) presented 15 pairs of values obtained from 7 patients, W. B. MacDonald and T. Stapleton (*Acta paediat. (Stockholm)* **44,** *559 (1955)*) had 9 such pairs obtained from 2 patients, and I. M. Dawson, W. S. Craig, and F. J. Perera (*Arch. Dis. Child.* **29,** *475 (1954)*) published 5 pairs obtained from one patient. A positive correlation was obtained from the total 51 pairs of calcium and cholesterol blood determinations made on 14 infants with idiopathic hypercalcemia. The coefficient of correlation was 0.72 with a standard error of 0.14, which indicates a significant positive correlation between the serum calcium level and the plasma cholesterol. These investigators speculate that the disordered metabolism in idiopathic hypercalcemia might be related primarily to an aberration in cholesterol metabolism. The factor or factors which initiate the sequence of events leading to idiopathic hypercalcemia are not known.

Recently W. M. Fyfe (*Lancet* **I,** *610 (1956)*) found that infants with idiopathic hypercalcemia have abnormal plasma vitamin A levels and react differently from normal individuals to a vitamin A load test. He determined the plasma vitamin A level, using a modification of the spectrophotometric method of O. A. Bessey, O. H. Lowry, M. J. Brock, and J. A. Lopez (*J. Biol. Chem.* **166,** *177 (1946)*), in blood obtained from infants with idiopathic hypercalcemia and from 7 healthy children (ages 4 to 14 months). Two of the latter infants had renal acidosis. These children were studied before and four hours after receiving orally a vitamin A test dose of 12,500 I.U. per kilogram of body weight, but the dose never exceeded 250,000 I.U.

The patients were on normal diets and all vitamin A concentrates were withdrawn for at least forty-eight hours before the test was performed. The concentrate used as a test dose consisted of a synthetic vitamin A palmitate in arachis oil. Normal children had an average pretreatment plasma vitamin A level of about 130 I.U. per cent, with the range being 40 to 200 I.U. per cent. Four hours following the administration of the test dose, the plasma vitamin A rose to a level of about 648 I.U. per cent with the range being 190 to 1255 I.U. per cent. The patients with idiopathic hypercalcemia had higher pretreatment plasma vitamin A levels than the normal children, averaging 275 I.U. per cent, with a range of 155 to 400 I.U. per cent. Following the test dose the vitamin A level of the plasma increased further to a mean level of 1786 I.U. per cent with a range of 600 to 3530 I.U. per cent. In spite of the fluctuations in the vitamin A levels of the plasma in both groups of children, those with the idiopathic hypercalcemia had definitely higher levels both before and after receiving the vitamin A load.

The vitamin A level of plasma is quite constant, according to H. C. S. Aron (*Am. J. Dis. Child.* **77,** *763 (1949)*), in spite of wide variation in vitamin A content of the diet. In healthy infants hyperalimentation with vitamin A for several weeks will cause an increase in the blood level which returns to normal within a few days after resumption of the normal diet. Since infants in this study did not receive vitamin A supplements for at least two days before beginning the study, vitamin A overdosage could not be responsible for the elevated plasma levels. Also, the increased plasma vitamin A concentration both before and after the test load could not be due to the malnourished condition of the infants, since it has been shown by A. B. McCoord *et al.* (*Pediatrics* **2,** *652 (1948)*) that undernourished infants have lower plasma vitamin A levels and also do not show large increases in the levels after a test dose. Fyfe *et al.* confirmed this, studying 5 infants with marasmas (progressive wasting and emaciation, of unknown origin). Thus in addition to abnormalities in calcium and cholesterol metabolism, infants with idiopathic hypercalcemia also show pecularities in vitamin A metabolism.

The etiology of idiopathic hypercalcemia was originally believed to be increased sensitivity to vitamin D. While the elevated serum calcium levels, increased rate of absorption of calcium from the intestinal tract, increased retention of calcium, hypercalcemia, azotemia (excess of nitrogenous compounds in the blood) and lowered serum alkaline phosphatase are all factors which are compatible with an increased vitamin activity in this syndrome, the vitamin D intakes of certain infants who have had this syndrome have been known to be low, and two different groups of investigators have found that addition of calciferol to the diet of these infants does not cause any significant clinical change. In addition, the calciferol does not cause an increased renal excretion of calcium. Following vitamin D intoxication in infants, recovery normally takes place within a month or six weeks after the withdrawal of the vitamin. This re-

covery period is considerably shorter than that noted in idiopathic hypercalcemia. Furthermore, the following mitigate against this disorder being due to increased vitamin D activity, namely, the increased retention of phosphorus, and the hypocitremia and hypocituria (lowered blood and urine levels of citrate).

Other investigators have suggested that cow's milk used in making the infants' formulas, because of its high calcium content, might be an inciting factor. Other factors must play a major role in causing this disorder, since only a very small number of infants on cow's milk formulas show this syndrome. Moreover, one of the patients of Morgan *et al.* was still being breast fed when the idiopathic hypercalcemia was noted. This infant received only small amounts of cow's milk in puddings and some cereal foods.

Another difficulty in attempting to determine the etiologic agent responsible for this disorder is its peculiar geographic distribution. It appears mainly in England and Northern Ireland, being rare in Sweden and apparently absent in both the United States and Canada. An adequate explanation of this disorder would have to suggest the reason for its rather well defined geographic limitations.

Forfar and associates suggested that the condition may be due primarily to an abnormality of cholesterol metabolism, in which a hypercalcemic but toxic product is being produced. This would explain many of the signs and symptoms, particularly why the clinical symptoms may not be worse when the serum calcium level is highest and vice versa. The reason why the disease apparently disappears in the benign cases is also not explained by this or any previous theory. Perhaps this may be a nutritional disorder caused primarily by overcorrection of some specific nutrient, producing a nutritional imbalance, as has been shown under rather rigid experimental conditions for certain vitamins and amino acids.

ADRENAL RESPONSE TO STRESS

The response of the adrenal cortex to a variety of stresses has been the subject of numerous articles (*Nutrition Reviews* **13,** *263* (*1955*)). Active investigation has continued in defining the role of these secretions in the metabolic patterns observed during stress (*Ibid.* **13,** *251* (*1955*)). In a further study of the metabolic response to surgery, R. W. Steenburg, R. Lennihan, and F. D. Moore (*Ann. Surg.* **143,** *180* (*1956*)) have attempted to correlate alterations in blood steroids with the phases of the postoperative response. It is necessary to observe blood steroid levels continually, as the rate of excretion of 17-hydroxycorticosteroids is correlated with the glomerular filtration rate as well as with the rate of secretion. In the postoperative period there are significant changes in glomerular filtration which may falsely alter the calculated secretion rates of the 17-hydroxycorticosteroids.

The authors studied 10 patients, noting eosinophil counts, 17-ketosteroid excretion, 17-hydroxycorticosteroid excretion and serum-free 17-hydroxycorticosteroids. Changes in these parameters of adrenal function were correlated with the sodium, potassium and nitrogen balances.

The eosinophil patterns were similar to those often reported. In all instances, the level was low at the time of operation and a low level was always correlated with a rise in serum 17-hydroxycorticosteroids. The urinary excretion of 17-ketosteroids did not significantly change as a result of surgery. The urinary total 17-hydroxycorticosteroids was elevated on the day of surgery and usually for two days thereafter. It should be noted that the operation

of greatest severity in this group was a subtotal gastrectomy.

The changes in serum-free 17-hydroxycorticosteroids were of greater significance. The rise was always early and steep, the peak levels being noted as early as four hours after the start of administration of anesthetic. The return to normal serum levels of 17-hydroxycorticosteroid was equally rapid, normal levels being reached by forty-eight hours after anesthesia. The early occurrence and high peak suggest a prompt adrenal response and tend to rule out the theory that the adrenal gradually responds to stress. This increased 17-hydroxycorticosteroid secretion is not necessarily correlated with sodium retention, which may continue during the descending curve of 17-hydroxycorticosteroid secretion.

It was noted that during the period in which serum 17-hydroxycorticosteroid levels have returned to normal, the negative nitrogen balance may still be present.

When ACTH was given intravenously in the postoperative period, there was a higher blood level of 17-hydroxycorticosteroids than when the ACTH was infused before surgery. Many mechanisms may be suggested to explain this. As examples, 25 units of ACTH given over an eight-hour period is not a maximal stimulus. Trauma alters the adrenal, making it more responsive to subsequent ACTH stimulation. There is a lesser excretion of 17-hydroxycorticosteroids because of decreased filtration rate. There is a slow rate of conjugation in the postoperative patient. The first possibility is unlikely, because an increase to 50 units of ACTH over the same period of time does not change the response. The second possibility cannot be ruled out by these studies. The authors feel, however, that there is a decreased conjugation of 17-hydroxycorticosteroids after trauma due to transient depression of hepatic function. In support of this, they note that the increased urinary excretion of conjugated steroids persists in some cases for one or two days after return of the steroid blood level to normal.

This study cannot answer the question of the cause-and-effect relationship between increased adrenal 17-hydroxycorticosteroid secretion and the metabolic response to surgery. In several types of trauma, the increased negative nitrogen balance continues long after the serum 17-hydroxycorticosteroids have returned to normal and, as stated above, patterns of sodium retention cannot be correlated with the levels of 17-hydroxycorticosteroids.

The reasons for this last seeming inconsistency were investigated by B. Zimmermann, J. H. Casey, and H. S. Bloch (*Surgery* **39,** *161* (*1956*)), who measured urine levels of sodium-retaining corticoid before and after surgery. This material was essentially absent in urine obtained preoperatively, but following surgery increased dramatically. The obvious step of correlating aldosterone excretion with sodium retention in the postoperative period is under study and should clarify the mechanism of this postoperative sodium retention.

In a complementary study to the one first discussed, D. M. Hume, D. H. Nelson, and D. W. Miller (*Ann. Surg.* **143,** *316* (*1956*)) examined the changes in blood and urine levels of 17-hydroxycorticosteroids in a series of severely burned men. Five of these patients had burns extending over 45 per cent or more of their surface area and did not receive cortisone or ACTH. The plasma 17-hydroxycorticosteroid levels were slightly high or high normal, and five to fifty days after the burns occurred the mean level was higher than in those patients with less severe burns. The urinary 17-hydroxycorticosteroids tended to be elevated in those patients whose convalescence was slow.

It is important to note that these studies were not performed until four days after the burns were incurred. By this time, serum 17-hydroxycorticosteroid levels have

usually returned to normal after major surgery. There was no evidence of adrenal "failure" during prolonged convalescence and the stress of subsequent surgery during this period led to marked increases in serum 17-hydroxycorticosteroid levels. When the blood 17-hydroxycorticosteroid levels were compared between those men who died and those who were as severely burned but survived, no differences were noted.

It is apparent from these studies that profound alterations in adrenal cortical secretions occur as a result of stress. The temporal sequence of these, related to the metabolic changes, does not prove a causal relationship, but a finer analysis of the secretion of aldosterone and hydrocortisone during this period may go far towards establishing such a relationship.

PREGNANCY TOXEMIA IN SHEEP

Ovine pregnancy toxemia, also referred to as twin-lamb disease, ketosis, acidosis, preparturient paresis, and pregnancy disease, has long been recognized as a clinical entity of economic importance. Apart from an inconsistent association with a low plane of nutrition (*Nutrition Reviews* **12,** *14 (1954)*), the etiology and pathogenesis of this disease are obscure. In recent studies G. L. McClymont and B. P. Setchell (*Australian Vet. J.* **31,** *53 (1955)*) make a tentative identification of the disease as a hypoglycemic encephalopathy.

The study was conducted in western New South Wales in the winter month of August. Fifty pregnant ewes, 20 ± 4 days prior to lambing, and 10 nonpregnant ewes served as the experimental animals. Prior to the experiment they were grazed on a short leafy growth of green feed, largely barley grass, and were considered in good nutritional health.

Complete starvation for four and one-half days was used to induce pregnancy toxemia. Water was available to the ewes at all times. Jugular vein blood samples were taken at twenty-four-hour intervals. Blood samples were also taken when toxemia symptoms were first noted.

Thirteen toxemia cases developed. All these animals were carrying twins, making an incidence of 62 per cent of all ewes in the flock carrying twins. Symptoms were noted in some animals within forty-eight hours after feed was withdrawn. In general, all toxemic ewes exhibited the symptoms referred to as depression or suppression of responses to stimuli applied to ocular, auditory and olfactory receptors. These are typical of field cases of the disease. Muscular twitching, clonic seizures and disturbances in posture were also noted.

Body temperature remained within normal limits (99 to 103° F.) while pulse rate was invariably high (140 to 180 per minute). There was no appreciable increase in respiration rate (25 to 50 per minute). In all cases of pregnancy toxemia induced by starvation there was appetite failure, as evidenced by complete absence of response to offering of fresh green grass or water.

A close correlation was noted between the development of symptoms and decreases in the concentration of blood sugar. This is of particular interest since in the past many workers have drawn attention to the lack of any consistent relation between the blood sugar picture and pregnancy toxemia in sheep (J. R. Holmes, *Vet. Record* **65,** *299 (1953)*). Minimal blood glucose levels of 18.1, 21.2 and 22.2 mg. per cent were found in toxemia cases, in pregnant nontoxemic animals and in the nonpregnant animals respectively. Although the hypoglycemia of toxemia was statistically highly significant when compared with symptom-free animals, these workers noted secondary rises in blood sugar levels.

This would explain why random blood samplings of field cases of the disease might not reveal any consistent pattern.

Hyperketonemia has been reported as a constant feature of field cases of pregnancy toxemia in sheep and it was observed in late stages in the induced cases. However, this condition was not present when symptoms of toxemia first appeared. There was no indication of disturbance in serum mineral concentration at any stage of the disease in those animals with the induced cases.

Seven of the 13 ewes with this syndrome were slaughtered within two to four days after development of symptoms of toxemia. Kidneys were found to be normal, livers ranged from normal to typical yellow-fatty, and adrenals in all animals but one were normal. McClymont and Setchell feel that gross microscopic renal abnormalities, liver fat infiltration and adrenal enlargement often reported in field cases are probably not etiologic factors in the disease but are seen secondarily as a result of prolonged starvation or undernutrition.

The tentative conclusion is drawn that pregnancy toxemia in sheep is primarily, if not solely, due to a cerebral depression and eventual cerebral damage as a result of hypoglycemia. The authors point to the striking similarity between ovine pregnancy toxemia and insulin-induced hypoglycemic encephalopathy in man (H. E. Himwich, "Brain Metabolism and Cerebral Disorders," *Williams and Wilkins, Baltimore (1951)*) and in sheep (R. L. Reid, *Australian J. Agr. Res.* **2,** *146 (1951)*).

With the provisional conclusion that ovine pregnancy toxemia represents a hypoglycemic encephalopathy, G. L. McClymont and B. P. Setchell (*Australian J. Biol. Sci.* **9,** *184 (1956)*) became interested in determining whether the brain of sheep is similar to the brain of man with respect to glucose metabolism. Recognizing that acetate is a peripheral metabolite of importance in ruminants, they investigated whether the brain of sheep possessed the capacity to oxidize acetate. Essentially identical acetate values were found between blood taken from the carotid artery and the venous blood from the dorsal sagittal sinus of the brain, indicating that the sheep's brain failed to utilize the acetate in the circulation. The mean glucose uptake by sheep's brain, 6.2 mg. per 100 ml. of blood, was sufficient, assuming complete oxidation of the glucose, to account for the mean oxygen utilization of 4.8 volumes per 100 ml. of blood.

Despite the metabolic adjustments of the ruminant directed at volatile fatty acid utilization, minimal circulatory glucose availability, and reduced insulin sensitivity (R. L. Reid, *Australian J. Agr. Res.* **1,** *338 (1950)*), these results fail to reveal any adjustment in favor of acetate utilization by brain tissue in the sheep.

ACUTE NIACIN DEFICIENCY IN THE CALF

Unlike the adult ruminant, the young calf requires a dietary source of certain B-vitamins (*Nutrition Reviews* **2,** *151 (1944)*; **9,** *270 (1951)*). Because of its large size and sensitivity to vitamin deficiencies, the young calf is a useful animal for studying comparative nutritional requirements.

J. H. Hopper and B. C. Johnson (*J. Nutrition* **56,** *303 (1955)*) have studied an acute niacin deficiency in young male calves of various breeds. The calves were placed on experiment when they were 24 hours old. They were fed two different "synthetic milk" diets containing 0.17 and 0.10 per cent of tryptophan respectively (by calculation). Both diets contained 13¦ per cent solids, which consisted of hydrolyzed and vitamin-free caseins, methionine, gela-

tin, glucose, lard emulsion, and necessary minerals and vitamins. The amount of tryptophan in the diet was varied by changing the proportion of hydrolyzed casein to vitamin-free casein. Microbiologic assay showed the diets to be essentially niacin-free.

The milk diets were prepared in batches of 100 gallons at a time, which is indicative of the difficulties associated with such experiments. The "milk" was refrigerated until used, when it was warmed to 37° C. before feeding.

A total of 8 calves was used, including 3 control animals fed niacin. Two out of 3 calves fed the diet containing no added niacin and 0.17 per cent of tryptophan developed severe scours and loss of appetite as early as the second day. Oral administration of niacin resulted in prompt improvement and gain of appetite and growth. The third calf did not exhibit symptoms during a twenty-two-day trial period nor did 2 control calves. All 3 deficient calves excreted less than 1.4 mg. of total niacin (determined by microbiologic assay) in the urine per day as compared with over 3.6 mg. excreted by the 2 control calves.

Two calves were then given the diet containing no added niacin and only 0.10 per cent of tryptophan. Both animals developed severe symptoms of scouring, dehydration and loss of appetite on the second day and appeared very weak. One animal died on the third day, apparently because of acute niacin deficiency. The other calf was given niacin by injection which caused improvement within twenty-four hours. Administration of niacin was then stopped, and scours resulted in forty-eight hours. This was again prevented by niacin injection. A control calf was fed the same diet plus niacin and appeared normal throughout a twenty-day period. It is unfortunate that larger numbers of animals were not used in these studies and that weight changes were not reported for the 5 animals kept on trial for twenty-four days or longer. In spite of this criticism the data are adequate to warrant the conclusions drawn.

No abnormal symptoms such as black-tongue were seen in any of the deficient animals nor were any changes seen upon postmortem examination. Because of the unusually rapid development of the deficiency, changes in tissue histology would not be marked, as the authors point out. However, biochemical studies would have been instructive, as would have more data on the niacin stores of the newborn calf, which must be very low.

Previous studies from this laboratory showed that niacin deficiency could not be produced when calves were fed a 30 per cent casein diet with larger amounts of tryptophan (*Nutrition Reviews* **5,** *256 (1947)*). Obviously, therefore, tryptophan can spare niacin requirements in the calf just as it does in other animals (*Ibid.* **8,** *85 (1950)*), but no quantitative figures are available.

It may be concluded that under proper dietary conditions the young calf is very sensitive to niacin deficiency, developing symptoms in twenty-four hours (an unusually short time for appearance of a vitamin deficiency). This information is important in animal nutrition and offers possibilities as a basic research tool in biochemistry.

FUNCTION OF THE ESSENTIAL FATTY ACIDS

A definitive biochemical role for the essential fatty acids, at least in the same sense that we understand the function of many of the B-vitamins, is still lacking. The occurrence of the chemical double bond in these acids, coupled with the knowledge that many biologic oxidation-reduction reactions are dehydrogenations, has led some to

believe that the essential fatty acids are vital in hydrogen transfer reactions, yet there is little sound evidence to support this view.

G. O. Burr and A. J. Beber (*J. Nutrition* **14,** *553* (*1937*)) made the early observation that fat-deficient rats had an abnormally high metabolic rate. This was confirmed by H. M. Sinclair ("Biochemical Symposia," No. 9, Lipid Metabolism, *Cambridge University Press, Cambridge, pp. 80–99* (*1952*)), who concluded that this effect was due to the high rate of water loss through the skin of the deficient animals. More recently, H. O. Kunkel and J. N. Williams (*J. Biol. Chem.* **189,** *755* (*1951*)) have found an increased activity of cytochrome oxidase in fat-deficient rats, an enzyme importantly concerned with biologic oxidation and energy production in the mitochondria of the cell. P. D. Klein and R. M. Johnson (*Ibid.* **211,** *103* (*1954*)) have in fact reported that oxidative phosphorylation associated with oxidation of DPNH (reduced pyridine nucleotide) is decreased in fatty acid deficiency. This condition would then resemble the "uncoupling" of oxidation and phosphorylation caused by thyroxine and dinitrophenol with the consequent change in metabolic rate.

In an effort to localize the defect in biologic oxidation, P. G. Tulpule and J. N. Williams (*J. Biol. Chem.* **217,** *229* (*1955*)) have assayed the activities of certain oxidative enzymes in liver homogenates of normal and fatty acid-deficient rats. The rates of oxidation of DPNH, succinate, and reduced cytochrome *c*, as well as the extent of oxidative phosphorylation (ATP formation) associated with these oxidations, were measured. In addition, the antimycin A-sensitive factor (AASF) (V. R. Potter and A. E. Reif, *Ibid.* **194,** *287* (*1952*)), a factor responsible for cytochrome *c* reduction in the electron transport scheme, was estimated. Since a vitamin B_6 deficiency had been shown to accentuate the symptoms of fatty acid deficiency (Tulpule and V. N. Patwardhan, *Indian J. Med. Res.* **38,** *3* (*1950*)), the enzyme studies were conducted on animals having the individual or combined deficiencies.

Male rats weighing 70 to 80 g. were divided into six groups of 8 rats each. Diets included two adequate control diets fed at different levels, and diets deficient in essential fatty acids, vitamin B_6, or both. The experiments were continued for six weeks, and consisted, for the four experimental tests, of a four-week period of restricted food intake on the doubly deficient diet to produce the deficiency, followed by a two-week period of ad libitum feeding of the same diet alone or supplemented with methyl linoleate, vitamin B_6, or both.

The succinate oxidase activity of liver was unaffected by deficiencies of either essential fatty acid or vitamin B_6, even though the activities of AASF and cytochrome oxidase, which are parts of the succinate oxidase system, were significantly increased. The feeding of vitamin B_6 had no effect on the activities of these enzyme factors, but methyl linoleate feeding partially restored the cytochrome oxidase activity to normal. The phosphorylative efficiency (P:O ratio) associated with DPNH and reduced cytochrome *c* oxidation in liver was reduced in the deficiency condition. Since cytochrome *c* oxidation is a part of the more complex system of DPNH oxidation, the authors believe this indicates a single site for the defect in biologic oxidation due to fatty acid deficiency, namely in the phosphorylation process concerned with cytochrome *c* oxidation. Since vitamin B_6 deficiency seemed only to potentiate the metabolic effects of fatty acid deficiency, it was concluded that vitamin B_6 is required in the utilization of linoleate.

In spite of the definite effects on biologic oxidation and oxidative phosphorylation demonstrated in fatty acid deficiency, it remains difficult to assign a specific biochemical role to the essential fatty acids.

It is worthy of note, however, that lipids clearly play an important part in the interaction of the cytochrome components in biologic oxidation (A. P. Nygaard and J. B. Sumner, *J. Biol. Chem.* **200,** *723 (1953)*; S. W. Edwards and E. G. Ball, *Ibid.* **209,** *619 (1954)*), and that purified cytochrome oxidase is associated with a high content of lipid and phospholipid containing arachidonic acid (E. Stotz, M. Morrison, and G. Marinetti, *p. 401, see esp. pp. 413–415,* "Enzymes: Units of Biological Structure and Function," edited by O. H. Gaebler, *Academic Press, Inc., New York (1956)*). Thus essential fatty acids may be critical as a part of the lipid matrix in which interaction of the components of biologic oxidation occurs.

RUMINANT-LIKE DIGESTION IN A MARSUPIAL

Pregastric bacterial digestion of feedstuffs, such as occurs in ruminants, confers important benefits upon the animal. With increasing population pressures there are obvious merits in favor of a ruminant-type digestive apparatus in the colonizing of geographic areas not capable of supporting mammals lacking a pregastric digestive system.

Although the gross value of ruminant digestion in terms of nutrition products for human consumption is recognized, a study of its anatomic features as a basis for understanding ecology is a neglected area. In this connection the recent studies of R. J. Moir, M. Somers, and H. Waring are of interest (*Australian J. Biol. Sci.* **9,** *293 (1956)*). The herbivorous marsupial *Setonix brachyurus* (the Rottnest Island wallaby, or the "quokka") weighs 2 to 5 kg. and is known to survive well under relatively poor grazing conditions. Moir and associates set out to examine the digestive system of this animal to determine to what extent it resembles the digestive system of the ruminant.

The marsupial possesses a complex stomach, sacculated, with an esophageal groove by-passing the sacculated region. Unlike the rumen of sheep, in which the whole of the rumen, reticulum and omasum has a stratified squamous epithelium, the epithelium of most of the stomach of the marsupial is columnar with well-developed tubular glands.

Moir and associates report that many of the digestive features of the marsupial are similar to those of a ruminant such as the sheep. The large sacculated stomach and the relatively small cecum suggest that in these animals pregastric bacterial digestion is predominant, rather than the cecal fermentation of other herbivores. Fifteen morphologic bacterial types, comparable to those found in the sheep rumen, were present in the sacculated portion of the marsupial stomach. Microbial attack on both cellulose and starch was observed. The volatile fatty acids formed were absorbed much as occurs in the ruminant through the portal circulation.

The level of volatile fatty acids in the sacculated stomach of the marsupial varied with feeding conditions and ranged from 2 to 14 millimoles per 100 ml. of fluid contents. The pH varied from 8.0 to 4.6. The gas of the stomach had a high carbon dioxide content. Blood sugar levels ranged from 15.8 to 78.9 mg. per cent, which appears more variable than values in true ruminants but of the same order.

The average ratio of stomach contents to large intestine contents in the marsupial was about 6:1. This is essentially the same as it is in ruminants and the reverse of that seen in nonruminant herbivores. The ratio of stomach content to body weight (1:1.5 in the marsupial) is however considerably less than for the true ruminants (from 1:5 to 1:10).

The relatively high costs of conducting

studies with large ruminants is partly responsible for the lag in the accumulation of nutrition knowledge in animals with a pregastric digestive system. The similarity of the digestive process of this small grazing marsupial to that of the large ruminant should therefore be of particular interest to nutritionists working with the ruminants.

PEPTIDES IN CASEIN SYNTHESIS

It has been shown that plasma proteins provide little of the nitrogen utilized for synthesis of casein in the lactating animal and that those plasma proteins which are converted into casein are first degraded completely to amino acids (P. N. Campbell and T. S. Work, *Biochem. J.* **52,** *217 (1952)*; B. A. Askonas, Campbell, C. Godin, and Work, *Ibid.* **61,** *105 (1955)*). However, it has not been clear whether the mammary gland is unable to use peptides or whether it is unable to produce them by partial degradation of plasma proteins.

To elucidate this question, C. Godin and T. S. Work (*Biochem. J.* **63,** *69 (1956)*) have attempted to test the ability of the mammary gland of the goat to utilize some of the lysine-rich peptides produced by partial acid hydrolysis of casein.

A normal lactating goat was used both as the source of peptides and as the experimental subject. A partial acid hydrolysate of casein was fractionated on an ion exchange column according to the method of Askonas *et al.* (*loc. cit.*) and divided into five groups of peptides, one of which contained a particularly high concentration of lysine peptides. This latter group of peptides, containing 81 mg. of nitrogen, was dissolved in 10 ml. of water together with 32.7 mg. of radioactive lysine. The mixture was injected intravenously into the goat immediately after the animal was milked dry. Two hours later, the animal was milked dry again and the casein was separated from the milk. To determine if the injected peptide had been utilized directly for casein synthesis, a partial hydrolysis of the two-hour casein sample was carried out, the results presumably indicating whether the presence of lysine peptides in the blood had resulted in nonuniform labeling of the casein. The effluent from the ion exchange column was analyzed qualitatively by single dimension paper chromatography. Each group of lysine peptides was hydrolyzed and the lysine was isolated and crystallized as bis-dinitrophenyl-lysine, by the method of Askonas *et al.*, for comparison of radioactivity. The results of the radioactivity determinations indicated that injected lysine was uniformly distributed among the different peptides. This, the authors state, indicates that peptides circulating in the blood cannot be used for casein synthesis without prior degradation to amino acids.

Although the above conclusion seems reasonable in the light of present information on the mode of protein synthesis (for example, M. B. Hoagland (*Biochim. et biophys. acta* **16,** *288 (1955)*) presents evidence that the first step in protein synthesis is the carboxyl activation of individual amino acids rather than the formation of peptides), the experiments are vulnerable to several criticisms. In the first place, the amount of injected peptide (unlabeled) was so small that dilution alone would seem to present a barrier to accurate analysis. Furthermore, an experiment in which a radioactive lysine peptide was injected would have permitted extensive tracing of the whereabouts of these peptides at various time intervals. In the same experiment it could have been ascertained whether the injected peptides reached the mammary gland intact, how rapidly they were hydrolyzed and, by radioautograph, whether the

peptides penetrated the mammary cell walls and where they were localized within the cells.

It is to be hoped that the authors will continue their experiments for the solution of these important problems.

DIETARY FAT AND LONGEVITY

Knowledge that the high growth rate associated with high caloric intake may be accompanied by a shortened life span (*Nutrition Reviews* **4,** *218 (1946)*; **5,** *319 (1947)*) would appear to necessitate a revision of criteria for the optimum diet. For example, it has been shown that a high fat diet accompanied by a high growth rate results in decreased longevity (*Ibid.* **13,** *278 (1955)*). Recently, it was demonstrated by H. J. Thomasson (*J. Nutrition* **56,** *455 (1955)*; *Nutrition Reviews* **14,** *305 (1956)*) that different oils differ markedly in their growth-promoting ability as a consequence of their influence on caloric intake. In particular, butterfat induced a much higher growth rate in rats than did an otherwise identical diet containing rapeseed oil.

In a continuation of these experiments, H. J. Thomasson (*J. Nutrition* **57,** *17 (1955)*) studied the longevity of rats fed equivalent diets differing only in the type of fat which amounted to 50 Calories per cent of either butterfat or rapeseed oil. The rats fed the butterfat diet grew more rapidly than did those on the rapeseed oil, the greatest difference in rate of gain (40 per cent) occurring at 10 weeks. This difference was presumably due to the greater food consumption of the rats on the butterfat diet (14 per cent, including food wastage). Under these conditions, the rats fed butterfat had mean life spans of 545 days as compared to the mean life span of 669 days of those fed rapeseed oil. This difference in longevity applied also to the per cent of survivors at any time and to the life spans of the last surviving animals. Histologic examination revealed only small differences between the two groups. These consisted of a connective tissue lesion of the hind legs and an increase in connective tissue of the kidneys, both lesions occurring in both groups of rats but to a significantly greater extent in the butter-fed animals.

These differences in the two groups of rats do not appear to be sufficient to account for the differences in life span. Correlation of maximum body weights with life span of all animals in both groups appears to eliminate the greater size of the butterfat-fed animals as a factor in longevity. However, there was a highly significant correlation between the number of days for achievement of maximum weight and the life span. The reasons for the increased longevity of the rapeseed oil-fed animals, therefore, are still not clear.

These experiments emphasize the often neglected fact that, in diet, optimum is not invariably synonymous with maximum. Although the difficulties of carrying out such experiments in the case of human beings seem almost insuperable, these experiments on rats appear to merit some serious consideration for their implications for reducing diets and nutrition in general.

A NUTRITIONAL REQUIREMENT FOR BROMINE

Of the elements of the halogen group, the greatest nutritional interest has centered on iodine. The importance of iodine as an essential precursor for the formation of

thyroid hormone is familiar to all. Some evidence has been presented which suggests that fluorine may be an essential nutrient of the diet. The elaborate precautions taken to exclude fluorine from natural food products in dietary experiments have been discussed previously (*Nutrition Reviews* **12,** *156 (1954)*). However, the evidence that fluorine is an essential nutrient remains inconclusive.

The possible role of bromine in nutrition has been investigated. In the first of two papers, J. W. Huff, D. K. Bosshardt, O. P. Miller, and R. H. Barnes (*Proc. Soc. Exp. Biol. Med.* **92,** *216 (1956)*) have considered the action of bromine in preventing the growth retardation which occurs in mice fed a diet containing iodinated casein. Purified diets composed largely of cottonseed oil, soybean protein and dextrin plus vitamin and mineral supplements were fed for experimental periods of twelve to twenty-five days. The experimental diets contained either 2 or 4 per cent of iodinated casein. In some experiments succinylsulfathiazole was added to inhibit intestinal bacteria. In initial experiments it was found that whey partially reversed the growth inhibition occurring with iodinated casein feeding. The fact that the activity of the whey preparation could be demonstrated in whey ash led to a trial of dried sea salt as a source of trace elements. In amounts of 0.5 per cent of the diet the sea salt preparation had a growth-promoting effect. The elements known to be present in dried sea salts which were not represented in the dietary salt supplement were aluminum, boron, bromine, fluorine and rubidium. When salts of each of these elements were added to the diet, activity was found to reside in the bromine salts. The addition of 3.75 parts per million (p.p.m.) of bromine was growth promoting but the combination of linseed oil and bromine was required for a complete reversal of the inhibition of growth produced in mice by iodinated casein.

The observations of I. Abelin and G. Poretti (*Helvet. physiol. et pharmacol. acta* **10,** *C 7 (1952)*) may help to explain the role of iodinated casein in evoking bromine deficiency. These authors have reported that there is an increased uptake of bromine by the liver, kidney, adrenals, brain and blood by hyperthyroid rats.

In the companion paper, D. K. Bosshardt, J. W. Huff, and R. H. Barnes (*Proc. Soc. Exp. Biol. Med.* **92,** *219 (1956)*) have described a possible nutritional effect of bromine in chicks. The growth of chicks on a semisynthetic diet was compared with that obtained when various supplements were added. In the first experiment the addition of whey and sea salt caused a significant growth augmentation of small extent. As in the case of the mice fed iodinated casein, the addition of bromine (8 p.p.m.) was as effective as sea salts. Although the observation could be confirmed in two other hybrid strains of chicks, it was not demonstrable in one experiment using an unidentified strain of New Hampshire cockerels. It is apparent that the growth-promoting effect of bromine, which is of small magnitude, may not be evident in all experiments. Such failures may be indicative of a sufficient carry-over from mother to progeny to supply the nutritional needs during the experimental period.

The above experiments would seem to indicate that under conditions of rapid growth or increased metabolism deficiencies of bromine may occur on bromine-free diets which are nutritionally correctable. It would be of importance to correlate these observations with measurements of bromine content of the body under the conditions of supposed deficiency.

The ubiquity of trace amounts of bromine would make it unlikely that spontaneous bromine deficiency could occur in human beings but this problem deserves investigation. The role of bromine in intermediate metabolism remains to be determined.

NOTES

Letter to the Editor

Dear Sir:

I wish to point out an error in your review of the paper on "Vitamin D and Gastric Secretion" by D. C. Herting and H. Steenbock (*Nutrition Reviews* **14,** *253 (1956)*). Gastric secretions collected from vitamin D-deficient rats on rachitogenic diets were found to have a higher pH, indicating *less* acidity, than did those collected from rats on normal diets. Supplementation with vitamin D lowered the pH of the secretions to the values found on normal diets.

Furthermore, the volume of secretions from rachitic rats was significantly decreased. This factor, in conjunction with the decreased acidity, indicates a more substantial reduction in the total H^+ ion than is suggested by either of these criteria alone.

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American Board of Nutrition

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition, in April 1957, at Chicago, Illinois. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Applications may be obtained from the Secretary, Otto A. Bessey, Environmental Protection Research Division, Quartermaster Research and Development Center, Natick, Massachusetts.

Child Health Services in Developing Countries

A new journal of interest to those in the nutrition field, *The Journal of Tropical Pediatrics*, has appeared. According to the lead editorial this journal hopes to serve as a forum for discussion of all types of problems of child health in tropical and subtropical zones, with particular emphasis upon the preventive aspects. This editorial states that "it is hoped that the Journal may draw attention to the ignorance of basic knowledge of all aspects of tropical pediatrics. For example, little accurate information is available on such fundamental subjects as infant mortality rates, the causes of death in childhood and 'normal' standards of growth and development; while knowledge of the pathology of disease in tropical childhood is almost non-existent. . . ."

The lead article in the new journal has been written by Dr. Cicely D. Williams, Senior Lecturer in Nutrition at the London School of Tropical Medicine and Hygiene (*J. Trop. Pediat.* **1,** *3 (1955)*). Dr. Williams discusses briefly the history of "child welfare centers" and considers their logical organization and function. She points out that "All too frequently one sees other countries, where there is little medical treatment available for children, there are few hospitals and no general practitioners, the infant mortality rate is over 300, and the death rate in the 1 to 4 age group is comparatively even higher, and yet doctors, trained only on the British pattern set up 'Welfare Centres' and ordain that they should do 'Preventive Work' and then are surprised at the ensuing failures." The reference to British pattern here is, of course, only a polite bow to other "advanced patterns" and Dr. Williams might as well have said the United States pattern. In common with others who have visited various health centers, she points out that she has "seen a mother trailing dejectedly away from one of these centres. The six month old baby on her back, chubby and cheerful has been weighed, examined and commended. The three-year-old child in her arms was suffering from malnutrition and dysentery. She had been advised to take it for treatment to

a dispensary five miles away. Is it any wonder that she did not pay much attention to a lecture on washing her hands before eating; or that she did not relish the idea of another ten mile walk, a long wait in a crowded 'out-patients' in order to receive a few pills, unaccompanied by any advice on the feeding and care of the sick child who was so desperately in need of it?" Dr. Williams stresses that although the pattern of health services in England works very well, it developed as a part of a particular medical and public health environment in which other services developed at the same time. The child health services "cannot operate, and never were intended to operate, in independent sections." They succeed "because of the doctors and hospitals that also exist."

The first guide in establishing such services should be the needs of the people rather than slavish following of the pattern in other countries. The services should provide care for the mother as well as the child and cannot evolve successfully "without adequate facilities for the investigation, treatment, and supervision of sick children." The author points out that nutrition problems are among the most urgent. These are sometimes due to the lack of suitable foods but often result from lack of knowledge of the needs of the child and from misuse of available foods. She points out that in the latter case it is not advisable to put much faith in the distribution of milk, except as an emergency measure. Rather, it would be more advisable to spend time and energy in training personnel to teach the people how to make the best use of the available, or potentially available, foodstuffs, among which milk is especially valuable.

Various other aspects of child health services are discussed. Those with experience or interest in the developing countries will find this article provocative and interesting.

Zinc and Lactic Dehydrogenase

Zinc has been shown to be a dietary requirement for several species of animals (*Nutrition Reviews* **8,** *239* (*1950*); **11,** *277* (*1953*); **13,** *303* (*1955*)). Only catalytic quantities of this element are required in the diet, which suggests that it may function in enzyme systems in the body. In this connection it has recently been shown that zinc is a functional component of glutamic dehydrogenase and alcohol dehydrogenase (B. L. Vallee, S. J. Adelstein, and J. A. Olson, *J. Am. Chem. Soc.* **77,** *5196* (*1955*); Vallee and F. L. Hoch, *Ibid.* **77,** *821* (*1955*)). Vallee and W. E. C. Wacker (*Ibid.* **78,** *1771* (*1956*)) have presented evidence which indicates that zinc is also a component of lactic dehydrogenase. In these most recent experiments crystalline lactic dehydrogenase was isolated from rabbit muscle. The enzyme was found to contain zinc in high concentration and in proportion to the purity of the various preparations. Metal binding agents were found to inhibit lactic dehydrogenase, which suggests that zinc may be necessary for activity of the enzyme.

It is becoming increasingly evident that the trace elements are essential components of enzymes.

Recent Nutrition Books

Modern Nutrition in Health and Disease. Edited by Michael G. Wohl and Robert S. Goodhart. Lea & Febiger, Philadelphia, 1955. Pp. 1062. Price: $18.50.

The Safety of Artificial Sweeteners for Use in Foods. A Report by the Food Protection Committee of the Food and Nutrition Board. National Academy of Sciences—National Research Council Publication 386, Washington, D. C., August 1955. Pp. 10.

Dentistry—As I See It Today. A. Leroy Johnson. Little, Brown & Company, Boston, Mass. Pp. 54.

A Symposium on Inorganic Nitrogen Metabolism. Edited by William C. McElroy and Bentley Glass. Reprinted from Contribution No. 125 of the McCollum-Pratt Institute. The Johns Hopkins Press, Baltimore.

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The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

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